

Patent claims:

- Sub E1
- 1) Human-compatible monoclonal antibodies which are specific for human-CD28 and activate human T-lymphocytes of several to all sub-groups without occupancy of an antigen receptor of the human T-lymphocytes and thus antigen-non-specifically.
- Sub D1
- 2) Monoclonal antibodies according to claim 1 which are available through
- A) production of hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies by means of an immunisation with non-T tumour cell lines on which human CD28 is expressed,
  - B) if applicable, humanisation of the monoclonal animal antibodies available from the hybridoma cells pursuant to phase A through a biochemical or gene-technological exchange of constant components of the animal antibodies against analogous constant components of a human antibody or replacement of genes of the hybridoma cells corresponding to the components;
  - C) secreting of the monoclonal antibodies in hybridoma cell cultures and isolation of the monoclonal antibodies from it or production of the monoclonal antibodies by injection of the hybridoma cells into animals, for example mice, and isolation of the monoclonal antibodies from the body fluid of the animals.
- claim 1
- a
- 3) Monoclonal antibodies according to ~~claims 1 or 2~~, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being available through
- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH $\beta$ APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
  - b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells by means of polyethylene glycol,
  - c) cultivation of the transfected cells received in phase b,
  - d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
  - e) immunisation of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
  - f) removal of spleen cells of the mice immunised in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol,
  - g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells and
  - h) cultivation/sub-cloning of the selected hybridoma cells obtained in phase g.

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- 4) Hybridoma cells for the production of monoclonal antibodies according to ~~one of the claims 1 to 3~~ <sup>Claim 1</sup> which are available through the following procedural steps:
- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH $\beta$ APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
  - b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells by means of polyethylene glycol,
  - c) cultivation of the transfected cells received in phase b,
  - d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
  - e) immunisation of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
  - f) removal of spleen cells of the mice immunised in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol and
  - g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells.
- Sub  
D1/

- 5) Procedure for the production of monoclonal antibodies according to ~~one of the claims 1 to 3~~ <sup>Claim 1</sup> with the following procedural steps:
- a

- A) production of hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies by means of an immunisation with non-T tumour cell lines on which human CD28 is expressed,
- B) if applicable, humanisation of the monoclonal animal antibodies available from the hybridoma cells pursuant to phase A through a biochemical or gene-technological exchange of constant components of the animal antibodies against analogous constant components of a human antibody or replacement of genes of the hybridoma cells corresponding to the components;
- C) secreting of the antibody in hybridoma cell cultures and isolation of the antibodies from it or production of the antibodies by injection of the hybridoma cells into animals, for example mice, and isolation of the antibodies from the body fluid of the animal.

- 6) Procedure according to claim 5, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being produced in the following procedural steps:

- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH $\beta$ APr-1-neo vector following excision of the Sall-HindIII

fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

- b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells by means of polyethylene glycol,
- c) cultivation of the transfected cells received in phase b,
- d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
- e) immunisation of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
- f) removal of spleen cells of the mice immunised in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol and
- g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells.

claim 1

- 7) Use of monoclonal antibodies according to ~~one of the claims 1 to 3~~ for the production of a medicine for the therapeutic treatment of the human body.
- 8) Use according to claim 7 for the production of a medicine for the treatment of diseases with pathologically reduced numbers of CD4 T cells, in particular AIDS or following stem cell transplantations after chemotherapy of leukemic diseases.
- 9) Use according to claim 7 for the production of a medicine for the potentiation and/or qualitative influencing of immune reactions in protective inoculations.
- 10) Use according to claim 7 for the production of a medicine to influence the quality of the T cell reaction; in particular to influence the production of various effector molecules, for example cytokines and chemokines and their receptors, for example in auto-immune diseases and AIDS.

claim 1

- 11) Use of monoclonal antibodies according to ~~one of the claims 1 to 3~~ for the treatment of diseases of the human body.
- 12) Procedures for the therapeutic treatment of the human body with monoclonal antibodies according to ~~one of the claims 1 to 3~~ being used.

add B1

Add 9.1